

# Effects of NaCl stress on seed germination, leaf gas exchange and seedling growth of *Pteroceltis tatarinowii*

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**Abstract:** Effects of NaCl on seed germination, leaf gas exchange and seedling growth were investigated. The germination of *P. tatarinowii* seeds was delayed in the presence of NaCl, and was significantly inhibited when NaCl concentration exceeded 17 mM ( $1.0 \text{ g}\cdot\text{L}^{-1}$ ). NaCl treatments induced reductions of transpiration rate, stomatal conductance and net photosynthetic rate and their reductions were significant with the increasing of NaCl concentration. However, intercellular  $\text{CO}_2$  concentration in seedling leaves under salt stress was significantly higher than that of controls. There was a significant reduction in survival, basal diameter increment and height increment with increasing NaCl concentration 50 days after NaCl treatment, and a significant reduction in the dry weight increment of roots, stems, and leaves was also observed in the NaCl treatments. The deleterious effect on the biomass increment was more evident in the case of stems and leaves. Results from this study suggest that *P. tatarinowii* is sensitive to salinity stress and the salinity threshold for seedling growth might be less 34 mM ( $2.0 \text{ g}\cdot\text{L}^{-1}$ ).

**Keywords:** Salt stress; Hoagland nutrient solution; Germination rate; Net photosynthetic rate; Survival; Biomass increment

**CLC number:** Q945.78

**Document code:** A

**Article ID:** 1007-662X(2006)03-0185-04

## Introduction

The presence of high concentrations of  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{Mg}^{2+}$  and  $\text{SO}_4^{2-}$  ions in saline soils inhibits growth of many plants. On the world scale, there is an area of around 380 million  $\text{hm}^2$  that is potentially usable for agriculture, but where production is severely restricted by salinity. The problem of saline soils is ever-increasing, due to poor irrigation and drainage practices, expansion of irrigated agriculture into arid zones with high evapotranspiration rates, or land-clearing, which leads to rising saline water tables. World-wide, 5% of the arable land ( $100\times 10^6 \text{ hm}^2$ ) is adversely affected by high salt concentration, which reduces crop growth and yield (Lambers 2003). Salinity is also a major environmental problem in China. Totally there is saline soil of about  $27\times 10^6 \text{ hm}^2$  in China, of which coastal shoal land accounts for 8% (Ma *et al.* 2000). Ward *et al.* (2003) indicated, growing plants that have deeper roots, and use more water than the current shallow-rooted annual crops would provide a long-term solution for managing the dryland salinity problem. *Pteroceltis tatarinowii* is a species of Elm (*Ulmaceae*) with deeper root system, and its bark is the main raw material for manufacturing Xuan paper, which is the famous traditional handwriting paper in China.

*P. tatarinowii*, a deciduous and a mid-fast-growing tree species, only grows naturally in China and is mainly distributed in mountainous limestone regions, along river and stream banks in Anhui, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Hebei, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Liaoning (Dalian), Qinghai, Shaanxi, Shandong, Shanxi, Sichuan, and Zhejiang provinces (Fang 1996). It is one of the China's first class pro-

tected tree species and an indicative plant of limestone soil. Liu *et al.* (1985; 1986) reported that the bark quality has a direct influence on the quality of Xuan paper. Now more paper-making factories want to buy the bark harvested from limestone soil. However there are not enough *P. tatarinowii* plantations growing on limestone soils. Therefore, greater demands for bark production will be placed on the new plantations of *P. tatarinowii*. To solve these problems, Chinese researchers have studied the seed source selection (Fang *et al.* 1998), seedling culture techniques (Fu *et al.* 2001), silvicultural practices (Fang *et al.* 2001), productivity and bark quality of *P. tatarinowii* (Fang *et al.* 2004; Li *et al.* 2001) and other related techniques (Fang *et al.* 2002; Fu *et al.* 2002). Published results indicate that the intensive management and cropping techniques employed with plantations of *P. tatarinowii* require an understanding of its site requirements, planting density and coppice systems. To our knowledge, no information is available on the salt tolerance for *P. tatarinowii* at different stages of its life cycle. The objective of this study is to investigate the effects of NaCl concentrations on seed germination, leaf gas exchange and seedling growth of *P. tatarinowii* so as to provide some fundamental bases for the site selection and plantation silviculture.

## Materials and Methods

### Seed materials

Seeds of *P. tatarinowii* were collected in October 2002 from Qingyang County, Anhui Province, China. The seed tree was about 25 m tall and 65 cm in diameter at breast height. After drying in open sunlight and removal of unwanted materials, the seeds were put into an open plastic box in the laboratory. The seeds were stratified in pails according to the size ratio of 1 (seed) to 3 (sand) under natural conditions in order to break seed dormancy. The stratification was started on Jan. 3, 2003 until the commencement of the experiment.

### Experimental design and treatments

The effects of six concentrations of NaCl on seed germination

**Foundation project:** This paper is supported by the National Natural Science Foundation of China (the grant number No.39970608).

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**Received date:** 2006-02-28; **Accepted date:** 2006-06-15

**Responsible editor:** Zhu Hong

of *P. tatarinowii* were examined in a germination chamber with a relative humidity between 60% and 80%, a temperature of 25 °C at day and 20°C at night, a photoperiod of 14 h of light and a photosynthetic photon flux density of 350  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (fluorescent tubes). Seeds were surface sterilized with sodium hypochlorite solution of 10% and then washed several times with sterile distilled water. Seeds were germinated on absorbent cotton media containing 0.0, 17.0, 34.0, 51.0, 68.0, and 85.0 mM of NaCl (equals to 0, 1, 2, 3, 4 and 5  $\text{g}\cdot\text{L}^{-1}$  of NaCl) with four replicates. Each replicate contained 100 seeds, and totally 2400 seeds were used. The germination was examined and recorded every day for each treatment. Seeds were regarded as germinated when the radicle was at least equal to half of the seed length. The seeds germinated were taken out when germination was recorded each time. After 26 days, the seeds, which did not germinate, were counted and dissected to identify the viable seeds and non-viable seeds. The germination was calculated according to the following formula:

$$G = \frac{N}{M} \times 100\% \quad (1)$$

where,  $G$  is the germination rate,  $N$  the number of germinating seeds,  $M$  the member of viable seeds initiated

The effects of six concentrations of NaCl on seedling growth and photosynthetic indices of *P. tatarinowii* were examined in a greenhouse at Nanjing Forestry University. Seeds were sowed in containers with vermiculite after they were stratified. When the height of the seedlings was about 5 cm, they were transplanted into black plastic boxes with Hoagland nutrient solution. The size of the plastic box was 55 cm (length)  $\times$  35 cm (width)  $\times$  25 cm (height), and the thickness of the plastics was 8 mm.

NaCl treatment was started on August 5, 2003 and six treatments of NaCl were 0.0, 17.0, 25.5, 34.0, 42.5, and 51.0 mM of NaCl (equals to 0, 1.0, 1.5, 2.0, 2.5 and 3.0  $\text{g}\cdot\text{L}^{-1}$  of NaCl), respectively. Each treatment had three replicates and each replicate had 25 seedlings. Mean seedling size for different NaCl treatments were shown in Table 1. During the experiment, the nutrient solution was aerated every day and changed every 3 days since the seedlings were transplanted into it. Adding NaCl regulated the NaCl concentrations in the Hoagland nutrient solution.

**Table 1. Mean seedling size in control and different NaCl concentrations before treatments**

NaCl concentration (mM)	Height (cm)	Basal diameter (mm)	Biomass per plant (g)			
			Leaf	Stem	Root	Total
0 (CK)	26.5	1.6	0.173	0.095	0.074	0.342
17.0 (S <sub>1</sub> )	25.4	1.4	0.128	0.069	0.053	0.249
25.5 (S <sub>2</sub> )	27.1	2.0	0.224	0.184	0.180	0.588
34.0 (S <sub>3</sub> )	24.7	2.2	0.201	0.179	0.168	0.549
42.5 (S <sub>4</sub> )	27.7	1.8	0.211	0.115	0.069	0.396
51.0 (S <sub>5</sub> )	30.0	1.8	0.207	0.145	0.137	0.489

### Measurement and data analysis

Height growth of seedlings was recorded in 10-day intervals for each treatment. Basal diameter and biomass of the seedlings were only measured before salt treatment and 50 days after the treatment. 5 plants at each treatment were taken for biomass measurements and divided into roots, leaves and stems. Then dry

weight (dried at 70 °C for 24 h) were measured on each root, leaf and stem sample.

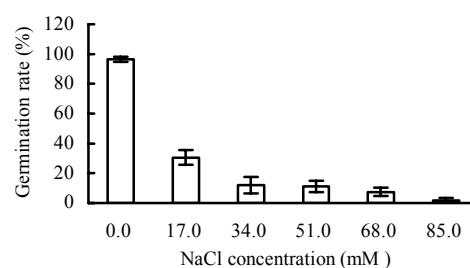
Leaf gas exchange measurements were conducted on five to eight medial leaves for each treatment 50 days after salt treatments. Transpiration rate ( $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), stomatal conductance ( $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), intercellular  $\text{CO}_2$  concentration ( $\mu\text{mol}\cdot\text{mol}^{-1}$ ) and net photosynthetic rate ( $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) were measured simultaneously with an LI-6400 Portable Photosynthesis System in the morning between 9:00 and 10:00 when the photosynthetic active radiation was over 900  $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

Data from this study were analyzed using SPSS 11.5 (Statistical Product and Service Solutions) software. After conducting an analysis of variance (ANOVA), the Duncan test was used to detect significant differences among the treatments with a probability of 95% ( $\alpha=0.05$ ).

## Results

### Effect of NaCl on seed germination

Seeds began to germinate at 8 days after sowing for the control (CK), while the seeds treated with 0.0, 17.0, 34.0, 51.0, 68.0, and 85.0 mM of NaCl began to germinate 16, 12 and 10 days after sowing, respectively. NaCl treatments greatly delayed seed germination. As depicted in Fig. 1, germination percentage of *P. tatarinowii* seeds with NaCl concentrations of 0.0, 17.0, 34.0, 51.0, 68.0, and 85.0 mM was 96.4%, 30.6%, 12.0%, 11.1%, 7.4%, and 1.9%, respectively, during the 26 days of the experiment. There was a significant reduction in germination of seeds ( $\alpha < 0.01$ ) with increasing NaCl concentration. Compared with the CK, 68.2% of the seed germination was reduced in 17.0 mM of NaCl treatment, while 87.6%, 88.5%, 92.3% and 98.0% of the seed germination were reduced in 34.0, 51.0, 68.0, and 85.0 mM of treatments, respectively. These results indicate that *P. tatarinowii* is sensitive to salt stress during the seed germinating stage, and its salinity threshold for seed germination is less 17.0 mM.



**Fig. 1 Germination rate of *Pteroceltis tatarinowii* seeds in 0.0, 17.0, 34.0, 51.0, 68.0 and 85.0 mM of NaCl at a temperature of 25 °C at day and 20°C at night 26 days after sowing (Means $\pm$ SD)**

### Effect of NaCl on leaf gas exchange

Salt stress significantly affected leaf gas exchange parameters of *P. tatarinowii* (Table 2). Salt treatments induced reductions of transpiration rate, stomatal conductance and net photosynthetic rate and their reductions were more evident with the increasing of NaCl concentration. Net photosynthetic rate of 17.0, 25.5, 34.0, and 42.5 mM of NaCl- treated seedlings was 95.7%, 40.6%, 27.5% and 5.8% of the control leaves 50 days after stress imposition, respectively (Table 2), while the mean net photosynthetic rate of leaves for 51 mM NaCl- treated seedlings was  $-0.8 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Stomatal conductance of 17.0, 25.5, 34.0, 42.5, and

51.0 mM of NaCl- treated seedlings was reduced by 18.0%, 38.6%, 59.4%, 64.8% and 82.5% with respect to that of controls, while the transpiration rate was decreased by 26.1%, 34.5%, 50.4%, 52.9% and 75.6%, respectively. However, intercellular CO<sub>2</sub> concentration in salt-treated seedlings was significantly higher than that of controls (Table 2). Compared with CK, intercellular CO<sub>2</sub> concentration of 17.0, 25.5, 34.0, 42.5, and 51.0 mM of NaCl- treated seedlings was 1.14, 1.17, 1.31, 1.60 and 2.14 times of the controls, respectively.

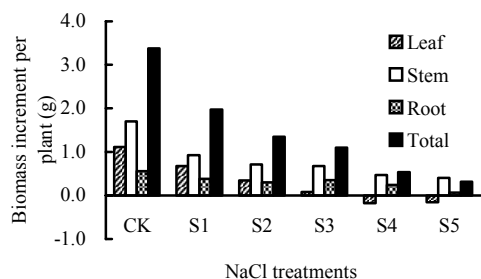
**Table 2.** Leaf gas exchange features 50 days after treatments of different NaCl concentrations (Values are the means±SD).

Treat-ments	Transpiration rate (mmol·m <sup>-2</sup> ·s <sup>-1</sup> )	Stomatal conduc-tance (mol·m <sup>-2</sup> ·s <sup>-1</sup> )	Intercellular CO <sub>2</sub> concentration (μmol·mol <sup>-1</sup> )	Net photosyn-thetic rate (μmol·m <sup>-2</sup> ·s <sup>-1</sup> )
CK	1.19±0.24a	79.5±16.7a	166.8±38.0cd	6.9±1.3a
S <sub>1</sub>	0.88±0.32ab	65.2±21.1ab	190.0±29.8bc	6.6±2.3a
S <sub>2</sub>	0.78±0.30ab	48.8±14.1bc	195.4±43.2bc	2.8±0.7b
S <sub>3</sub>	0.59±0.19bc	32.3±13.5cd	218.6±39.5bc	1.9±0.5bc
S <sub>4</sub>	0.56±0.19bc	28.0±11.1cd	267.7±37.8b	0.4±0.3cd
S <sub>5</sub>	0.29±0.13c	13.9±3.0d	357.0±33.4a	-0.8±0.4d

**Note:** Data were analyzed by Duncan's multiple range test and means followed by identical letters were not statistically different within the columns ( $P < 0.05$ )

### Effect of NaCl on seedling growth

The seedling of *P. tatarinowii* had optimal survival and growth in the nutrient solution without any added NaCl (Fig. 2, Table 3). Medium levels of salinity (17.0 and 25.5 mM of NaCl) did not cause substantial inhibition of growth but the general tendency was that increasing concentrations of NaCl induced a progressive decline both in survival and seedling growth. There was a significant reduction in survival, base diameter increment and height increment with increasing NaCl concentration (Table 3). The 50 days after the salt treatment, the survival of seedlings in CK obtained 91.8%, which was 14.8%, 21.6%, 246.4%, 410.0% and 1455.9% higher than that of S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub> and S<sub>5</sub> treatments, respectively. For the survival seedlings, the base diameter increment in S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub> and S<sub>5</sub> treatments was 83.3%, 70.0%, 36.7%, 60.0% and 36.7% of the CK, while seedling height increment was 87.2%, 83.5%, 52.8%, 40.2% and 55.6% of the CK, respectively.



**Fig. 2** Mean biomass increment of *Pteroceltis tatarinowii* seedlings in leaf, stem, root and total at 0.0 (CK), 17.0 (S<sub>1</sub>), 25.5 (S<sub>2</sub>), 34.0 (S<sub>3</sub>), 42.5 (S<sub>4</sub>) and 51.0 (S<sub>5</sub>) mM of NaCl concentrations 50 days after treatments

A significant reduction in the dry weight increment of roots, stems and leaves among treatments was observed 50 days after

NaCl treatment (Fig. 2). The deleterious effect on the biomass increment was more evident in the case of stems and leaves. For example, leaf biomass increment was negative in S<sub>4</sub> and S<sub>5</sub> treatments 50 days after NaCl treatment. Inhibition was much higher with the increasing of NaCl concentration and the total biomass increment per plant in S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub> and S<sub>5</sub> treatments was only 58.6%, 39.9%, 32.7%, 15.8% and 9.3% of the CK, respectively.

**Table 3.** Survival, mean increment of base diameter and seedling height after fifty days of NaCl treatments (Values are the means±SD).

Treat-ments	Survival (%)	Basal diameter (mm)		Seedling height (cm)	
		Mean	Increment	Mean	Increment
CK	91.8	4.6±0.9	3.0a	62.3±6.3	35.8a
S <sub>1</sub>	80.0	3.9±1.2	2.5ab	56.6±8.2	31.2a
S <sub>2</sub>	75.5	4.1±0.5	2.1b	57.0±8.4	29.9a
S <sub>3</sub>	26.5	3.3±0.3	1.1c	43.6±7.6	18.9b
S <sub>4</sub>	18.0	3.6±0.9	1.8bc	42.1±5.9	14.4b
S <sub>5</sub>	5.9	2.9±0.8	1.1c	49.1±9.1	19.1b

**Note:** Data were analyzed by Duncan's multiple range test and means followed by identical letters were not statistically different within the columns ( $P < 0.05$ )

### Discussion

Data of this experiment indicated that the germination of *P. tatarinowii* seeds was delayed in the presence of NaCl, and significantly inhibited when NaCl concentration exceeded 17.0 mM. Similar trends have been found in several species of *Atriplex*, in some annual halophytes (Noe *et al.* 2000; Debez *et al.* 2001) and in *Nicotiana tabacum* (Niknam *et al.* 2004). We found twenty-six-days salt treatment inhibited germination without damaging the seeds when the seeds, which did not germinate, were dissected to identify the viable seeds. Hence, the main factor involved in the salt induced dormancy of *P. tatarinowii* seems to be the low water potential of the medium, as described for *Carpobrotus* (Weber *et al.* 1999), *Suaeda fruticosa*, *Triglochin maritime* (Khan *et al.* 1997) and *Aeluropus lagopoides* (Gulzar *et al.* 2001). Our data confirm the assumption that salinity usually impairs germination by preventing imbibition. This result suggests that *P. tatarinowii* would be able to constitute viable seeds bank when salinity levels are high, and that these seeds would germinate after salt leaching from the soil surface by the rains.

Reductions in net photosynthetic rate appeared mainly limited by CO<sub>2</sub> diffusion into the leaves during salinity stress, as previously reported to occur in other plants (Tattini *et al.* 1997; Moya *et al.* 1999). The finding of the intercellular CO<sub>2</sub> concentration markedly increased in salt-treated seedlings as compared to control leaves (Table 2), suggesting that biochemical limitations to net photosynthetic rate might have prevailed over stomatal or mesophyll limitations 50 days after treatment of NaCl. These data strongly support the idea that biochemical limitations become prevalent in leaves with a massive salt load (Gucci *et al.* 1997; Allakherdiev *et al.* 2000). The significantly higher reduction in net photosynthetic rate than in stomatal conductance and transpiration rate, and a higher intercellular CO<sub>2</sub> concentration in NaCl treated seedlings (Table 2) suggest that stomatal inhibition of photosynthesis, caused by direct effects of NaCl on photosynthetic apparatus independent of stomatal closure, might be the major factor responsible for the drastic reduction in photosyn-

thetic rate in NaCl treated *P. tatarinowii*. Similar result was found in *Glycine soja* (Kao *et al.* 2003). However, our results were different from those in *G. tomentella* (Kao *et al.* 2003), *Phaseolus vulgaris* (Brugnoli *et al.* 1991) and spinach leaves (Downton *et al.* 1985), where the reduction of net photosynthetic rate was probably not caused by the ion toxicity but mainly due to the salinity modification of stomatal conductance.

In response to increasing concentration of NaCl in the culture medium, plants have to cope with the lowering of external water potential. Consequently, the reduction of plant growth is usually attributed to osmotic stress. Our results indicated salt stress significantly reduced seedling growth of *P. tatarinowii*, in agreement with those from eucalyptus (Morabito *et al.* 1996). Based on the fact that increasing concentrations of NaCl induced a progressive decline both in survival and seedling growth (Table 3 and Figure 2), the inhibition of the growth could be the result of ions, particularly sodium and chloride likely to be absorbed into the plant to adjust their hydric potential in response to the external solution. Unlike halophytes, glycophyte species seemed unable to compartmentalize ions which become toxic for the cellular metabolism (Greenway *et al.* 1980). *P. tatarinowii* did not depart from this behavior and salt tolerance of this species appeared to be related to a low level of salt accumulation in the leaf and stem compared to the root. The significant reductions in seedling growth at relatively mild external NaCl concentrations seem to indicate that *P. tatarinowii* is sensitive to salinity stress. And the results from survival and total 34.0 mM·L<sup>-1</sup> biomass increment suggest that the salinity threshold for seedling growth of *P. tatarinowii* might be less

## Acknowledgements

The authors are grateful to Dr. Hailong Wang from New Zealand Forestry Research Institute for his valuable comments and English corrections.

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